Adipose stem cells enhance excisional wound healing in a porcine model

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Contents

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✓relevant to surgical practice and teaching.

✓review articles

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Abstract

- Background
- Methods
- Results
- Conclusions

Introduction

➢ Adipose-derived stem cells (ASCs)

- popular candidates for cell-based therapies
- easily harvested
- cultured from liposuction fat
- in an outpatient setting with minimal risk of Complications
- large quantities harvested from a small amount of fat.

Introduction

➢Adipose-derived stem cells (ASCs)

- four or more passages without altering growth or differentiation
- can be expanded 3000-fold or more a few weeks
- 10 mL of fat to be expanded to 3 billion or more ASCs
- capable of replacing a number of tissues

  including bone, cartilage, muscle, and fat.

-

Introduction

➢Adipose-derived stem cells (ASCs)

- complex paracrine mediators of tissue repair and regeneration
- attract host regenerative cells to the site of injury.
- augmenting wound healing, particularly in difficult

injuries such as non healing wounds or burn wounds.

- uniform standards for dose and delivery are not yet established

Introduction

Rodent model

- differs from human skin both structurally and functionally

porcine model

- structurally similar to human skin
- employs similar repair mechanisms following injury.
- widely used in wound healing studies for more than 30 y.

Introduction

previous studies

Hadad et al

- improved wound vascularization and closure
- combination of ASCs and platelet-rich plasma

study aims

- Standardized model of cell dose and delivery

Materials and methods

1. Isolating and preparing ASCs
2. Tracking ASCs
3. Animals
4. Wounding and treatment
5. Dressing changes
6. Wound harvest and assessment
7. Real-time quantitative reverse transcriptase polymerase chain reaction
8. Western blots
9. Statistics

Materials and methods

Isolating and preparing ASCs

- porcine inguinal adipose → Allogeneic adipose stem cells

1. manually chopped
2. digested in double strength collagenase solution
3. shaking in a 37˚C water bath for 45-60 min
4. centrifuged for 10 min at 180 g
5. filtered using 2-ply gauze to remove large debris
6. cellular pellet was resuspended in erythrocyte lysis buffer

Materials and methods

Isolating and preparing ASCs

7.centrifuged at 180 g for 10 min → stromal vascular fraction

8. Falcon 175cm^2 tissue culture-treated flasks in general ASC culture medium

Dulbecco's Modified Eagle medium/Nutrient: F12

10% fetal bovine serum

1%penicillin/streptomycin

1% Fungizone

9. overnight incubation

Materials and methods

Isolating and preparing ASCs

10.Non adherent cells were removed

10. the cell medium was replaced with fresh plating medium

11. ASCs were then expanded in culture and passaged

12. 70%-80% confluency ⟶ lifted using trypsin

13. -80˚C in freezing medium: 10% fetal bovine serum

40% Dulbecco's Modified Eagle medium

10 % dimethyl sulfoxide

Materials and methods

Isolating and preparing ASCs

week of surgery

15.culture for 3 d to allow recovery

16. ASCs were lifted, counted, and assessed for viability

Tracking ASCs

1.ASCs were lifted and stained with PKH26

(a nontoxic fluorescent marker of cell membranes)

2. washing

Materials and methods

Tracking ASCs

3. sterile phosphate-buffered saline (1.3, 4.2, or 12.6 million cells/mL)

4. Aliquote into sterile 3 mL syringes

Animals

- Six-month-old (w70 kg) female Yorkshire pigs
- approval by the Institutional Animal Care.
- Committee of the University of Pittsburgh

Materials and methods

Animals

- housed in individual cages after wounding
- maintained under a 12-h light/dark cycle
- temperature in accordance with guidelines approved by the Institutional Animal Care and Use Committee.

Materials and methods

Wounding and treatment

- Two authors
- groups evenly distributed between them
- Forty (n = 8 per group) full-thickness circular wounds,
- 4 cm in diameter were created on the animals' dorsum using sharp excision
- A template was employed to standardize wound dimensions.
- Cells were injected at low, medium, and high doses

Materials and methods

Wounding and treatment

- The low dose: 0.3×10^6 ASCs/cm^2 (3.8×10^6 cells/wound)
- the medium dose: 1.0×10^6 ASCs/cm^2 (12.6×10^6 cells/wound)
- the high dose: 3.0×10^6 ASCs/cm2 (3.8×10^7 cells/wound)

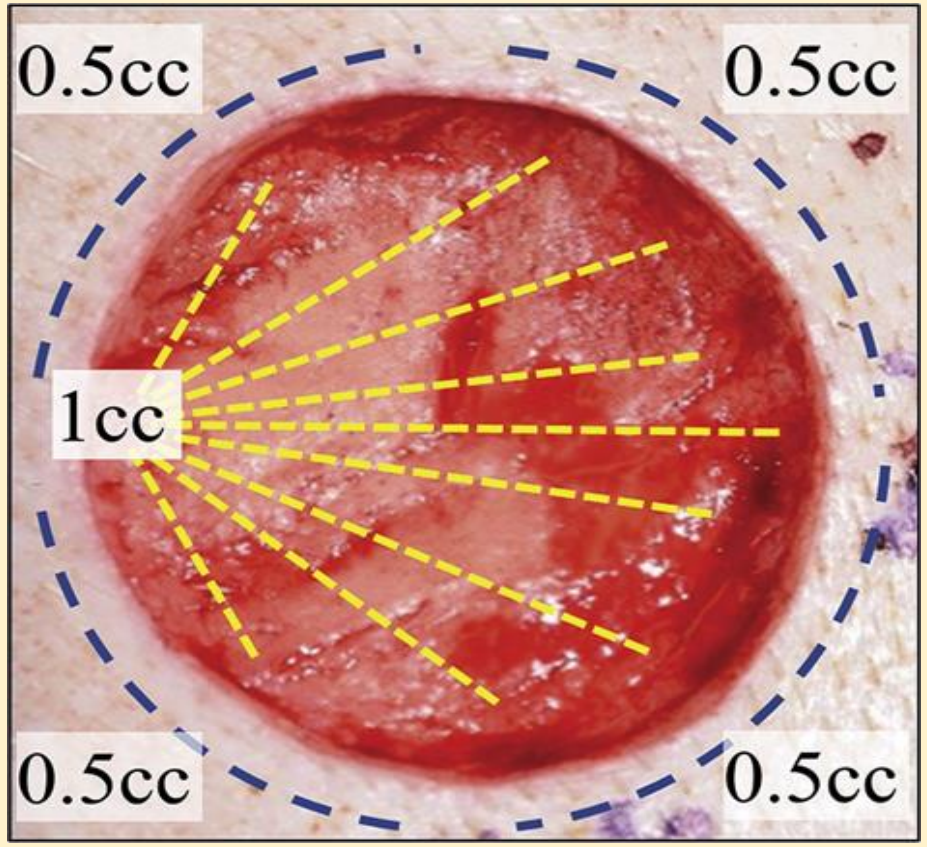
- Controls: sham injections of saline

  standard wound care without injection.
- Treatment locations were randomized

Materials and methods

Wounding and treatment

- Cell injections were distributed evenly around the wound
- 1 mL → superficial wound bed
- 2 mL → Intradermally
- Tegaderm dressings and OpSite
- A cotton pad and pig jacket to further protect



Materials and methods

Dressing changes

- twice weekly
- gentle debridement of fibrinous exudate
- saline rinsing as appropriate
- Photographed
- traced onto clear plastic to track contraction and epithelialization.
- Dressings were reapplied

Materials and methods

Dressing changes

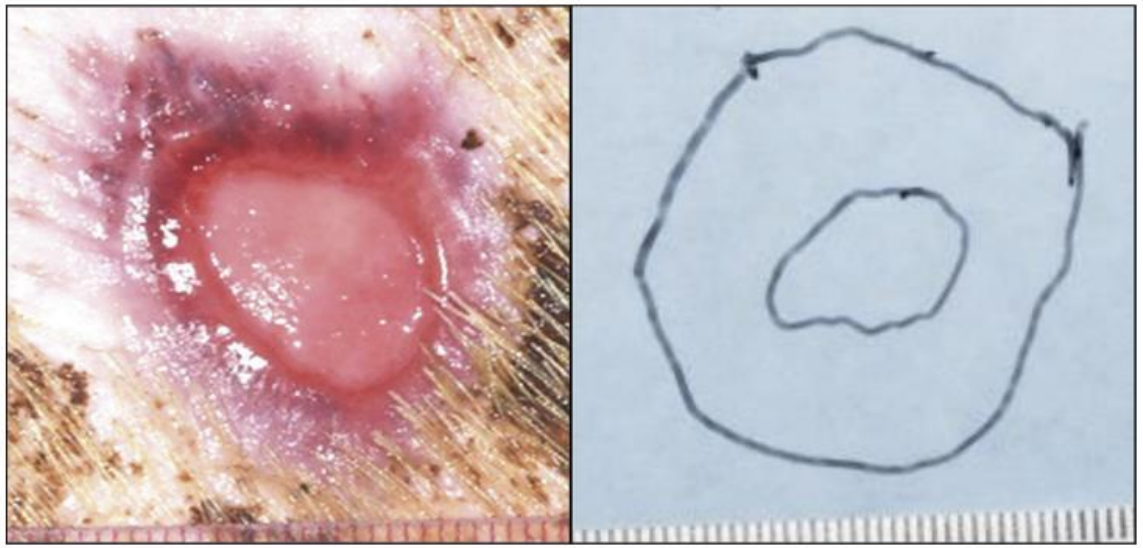


Fig. 2 – Wound contraction and epithelialization borders were traced onto clear plastic. Plastic tracings were photographed in a standardized fashion with a low distortion macro lens at a fixed distance. Contracted and epithelialized areas were calculated using ImageJ. (Color version of figure is available online.)

Materials and methods

Wound harvest and assessment

- Animals were sacrificed

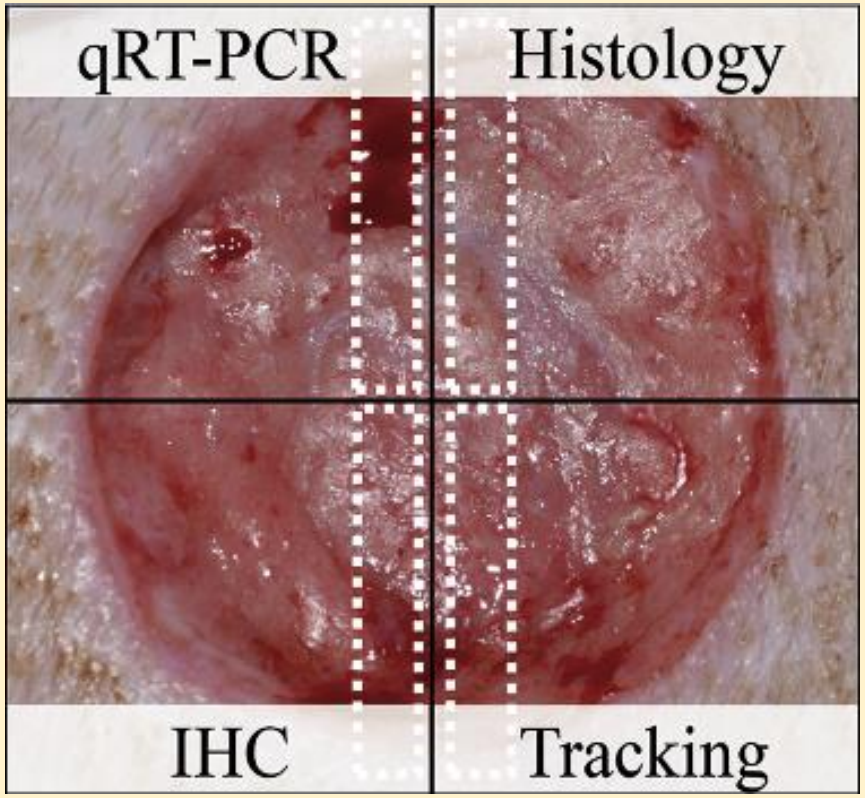
at 1 and 2 wk time points

- Wounds were excised to fascia
- Central wound were taken for histology
- Samples fixed:

➢Bouin's solution

or

➢frozen in Tissue-Tek O.C.T



Materials and methods

Wound harvest and assessment

- Masson’s trichrome stain
  - Basic tissue architecture
  - Neodermal thickness
- Fluorescent microscope :PKH-labeled exogenous ASCs.
- horseradish peroxidase :CD31 was assessed
- Metamorph image analysis software:

contraction and epithelialization were assessed

Materials and methods

Real-time quantitative reverse transcriptase PCR

- α-SMA(α-smooth muscle actin)
- Col1a1:Col3a1 ratio
- Samples were preserved in 900 μL of RNALater
- Stored at -20˚C
- homogenized and digested with 20 mg/mL Proteinase K
- RNA was eluted in RNase-free water

Materials and methods

Real-time quantitative reverse transcriptase PCR

- 500 ng of RNA was used as the template for cDNA
- final cDNA reaction was diluted 10x.
- 2µL was mixed with 5µL fast SYBR-green master mix.
- 3µL of primers mix specific to the target gene.
- The $2^{-\Delta\Delta ct}$ method

Materials and methods

Western blots

- CD31 and α-SMA.
- liquid nitrogen.
- lysis buffer on ice for 10 min.
- centrifugation at 12,000 g for 30 min.
- The supernatants were collected as whole cell extracts.
- lysates were concentrated by adding less lysis buffer

Materials and methods

Western blots

- protein concentrations. (bicinchoninic acid protein assay kit)
- polyacrylamide gel electrophoresis
- transferred onto nitrocellulose membranes,
- incubation with primary antibodies at 4˚C overnight
- washing
- HRP-conjugated secondary anti-bodies at room temperature for 1 h
- Proteins were detected with an enhanced chemiluminescence kit

Materials and methods

Western blots

- Loading control
  - ➢ Glyceraldehyde 3-phosphate dehydrogenase

Or

  - ➢B-tubulin
- Epson Perfection V600 photo scanner

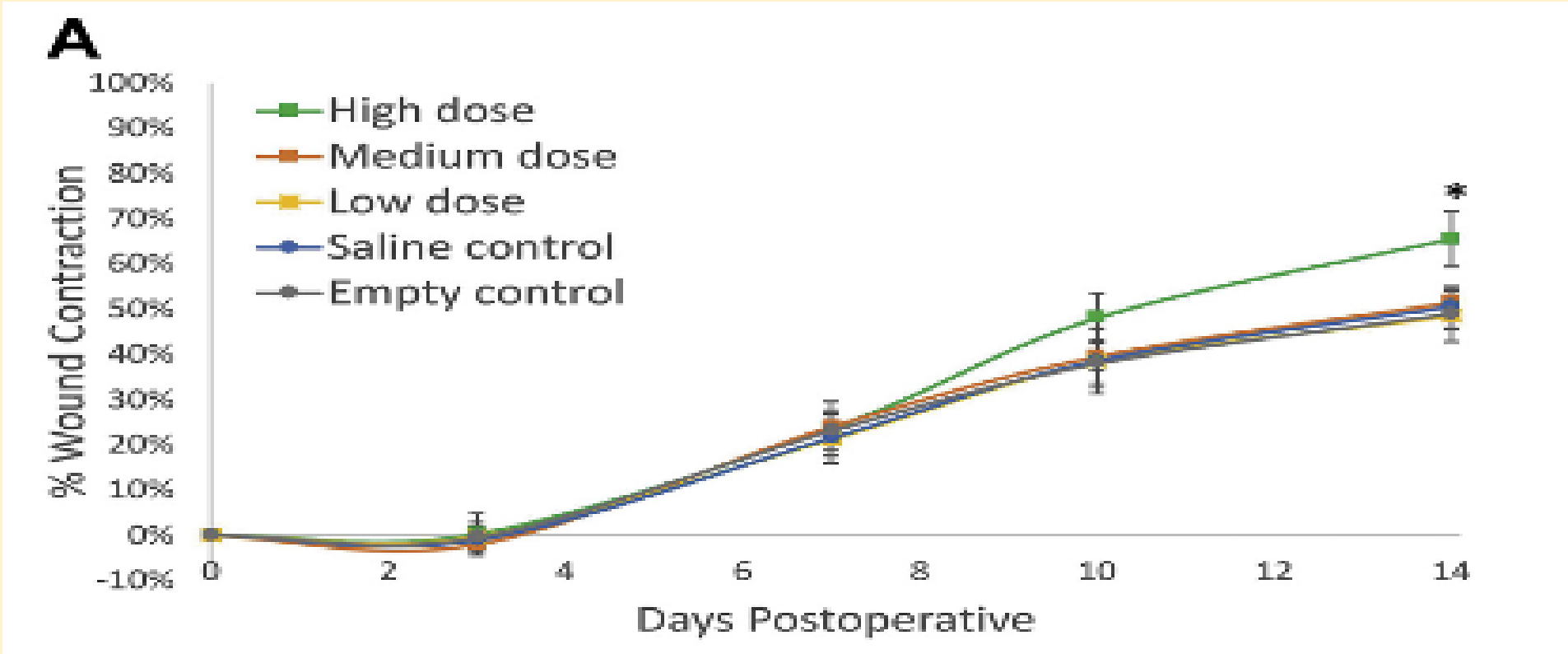
Materials and methods

Statistics

- Mean ± standard error of the mean
- P <0.05
- SPSS statistical software

Results

Wound contraction and dermal thickness



Results

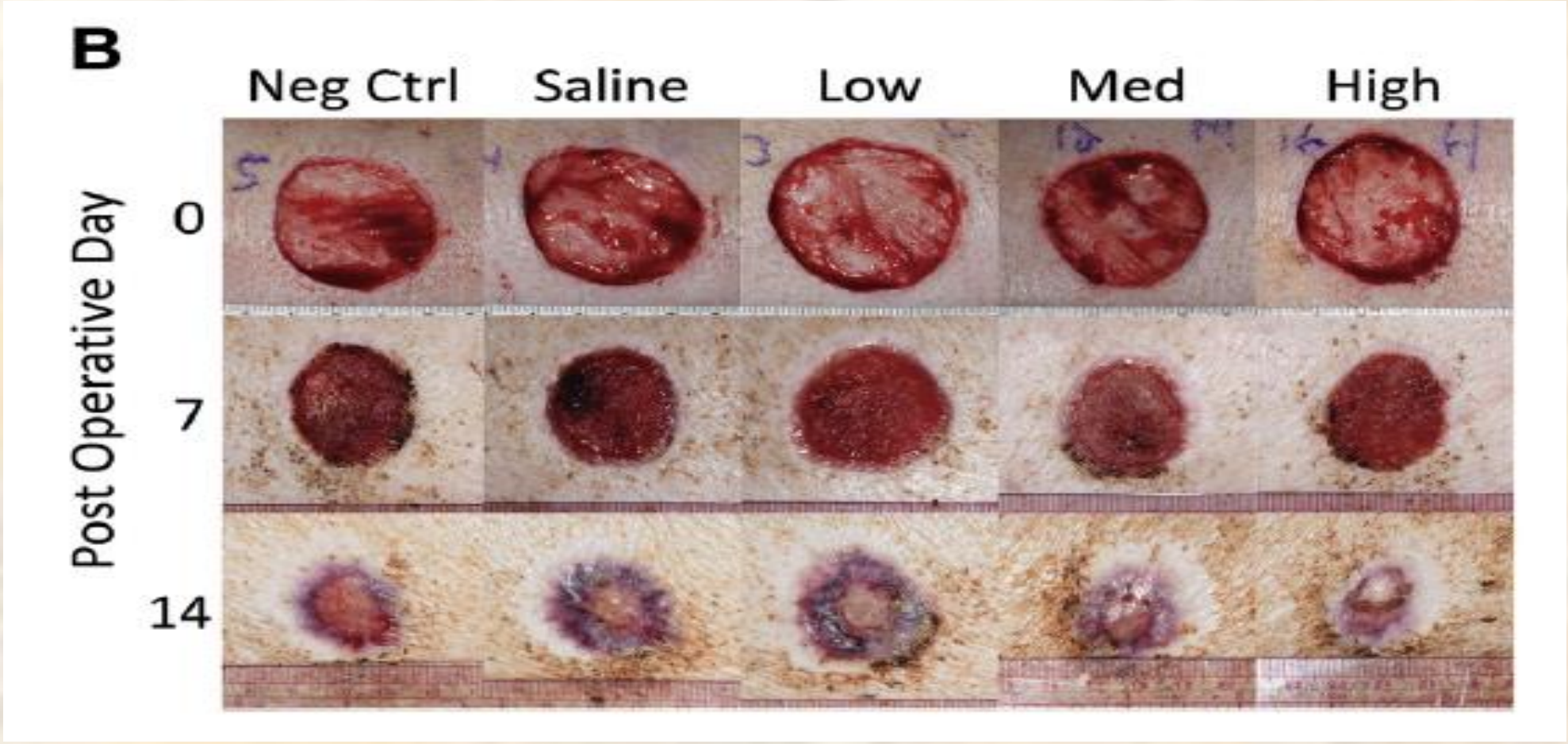
Wound contraction and dermal thickness

- %wound contraction:

original wound area -current wound area/original wound area

- High dose ASCs compared to saline injected controls:
  - greater wound contraction at day 10 ($p = 0.12$)
  - reached significance at day 14 ($p = 0.04$)

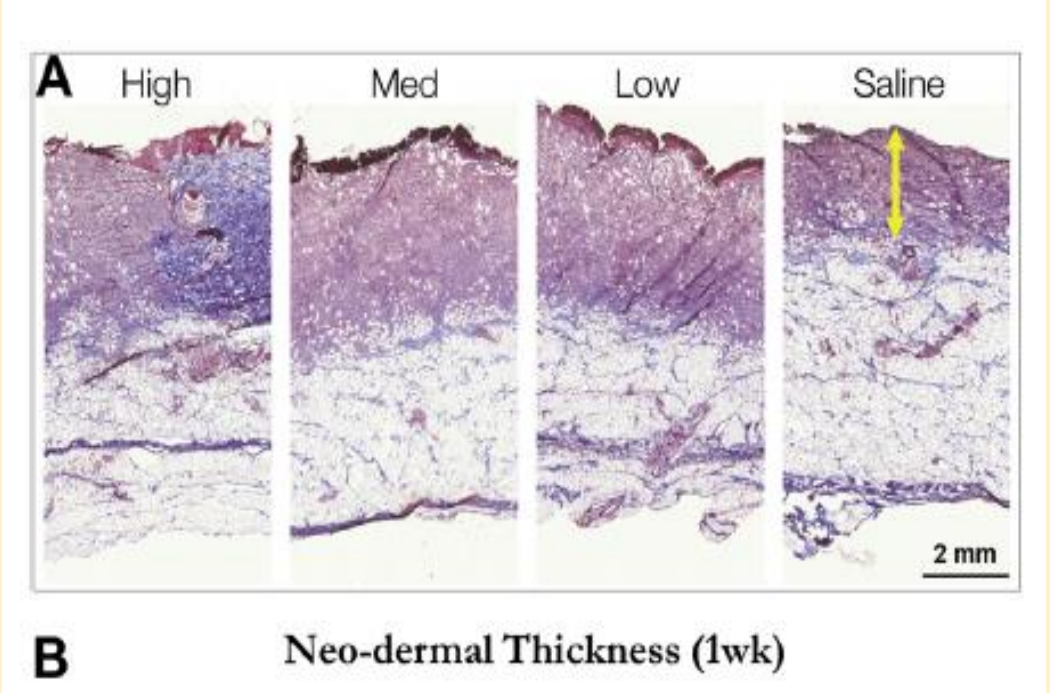
Results



Results

Wound contraction and dermal thickness

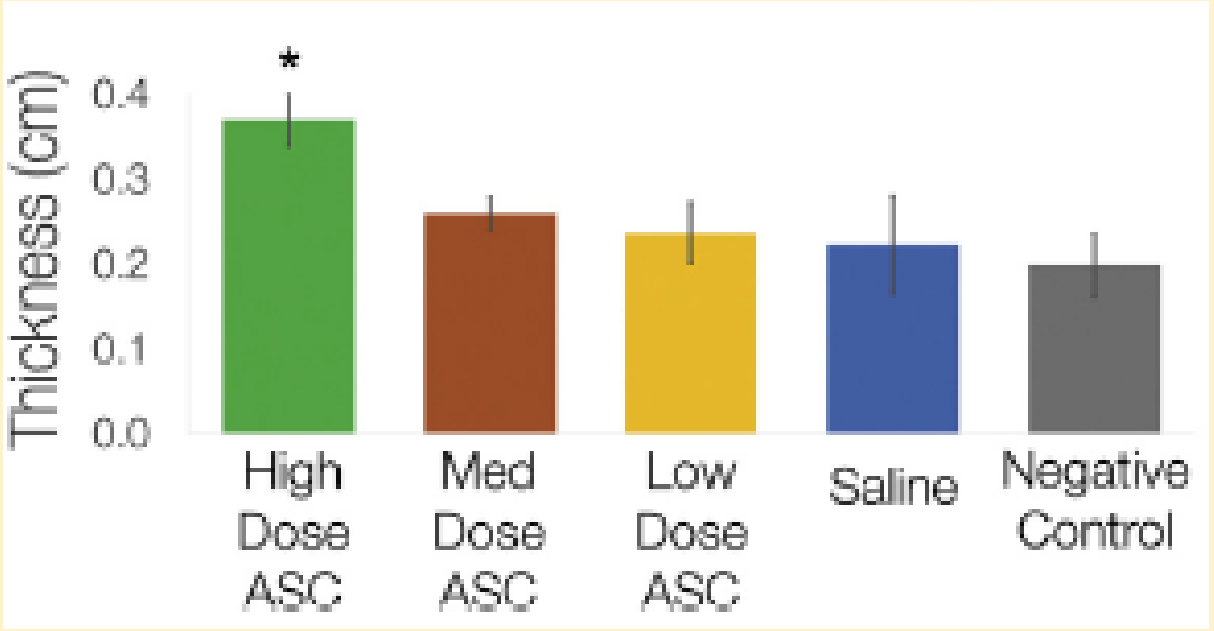
- Masson's trichrome at 1 wk postoperative



Results

Wound contraction and dermal thickness

- blinded observer



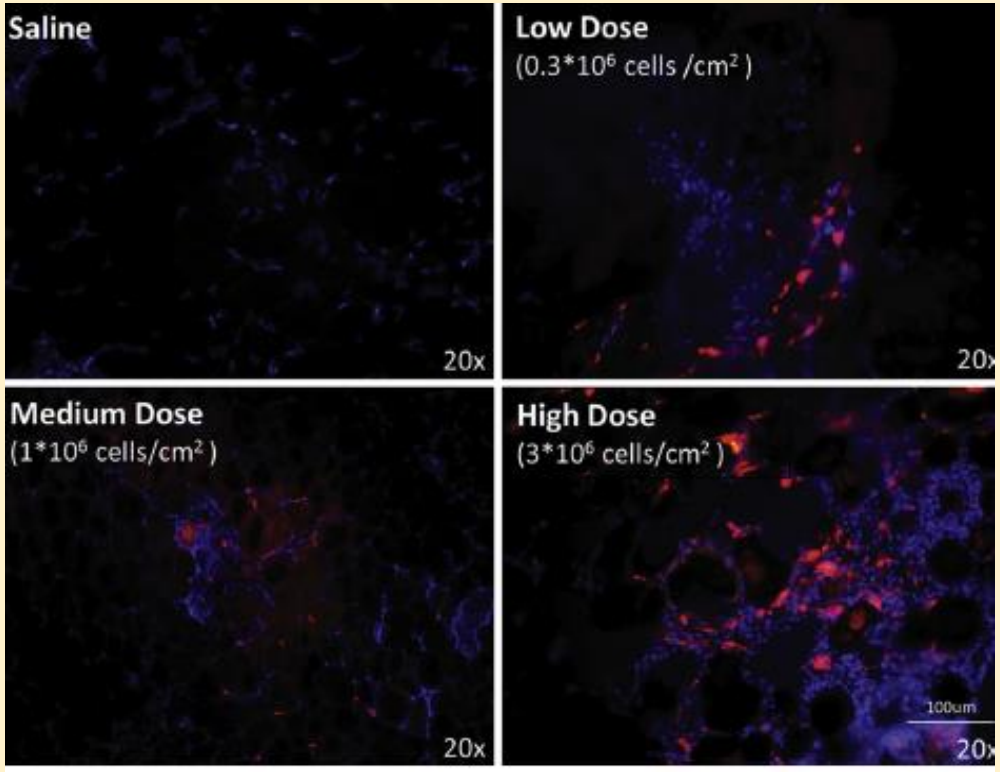
Results

Cell tracking

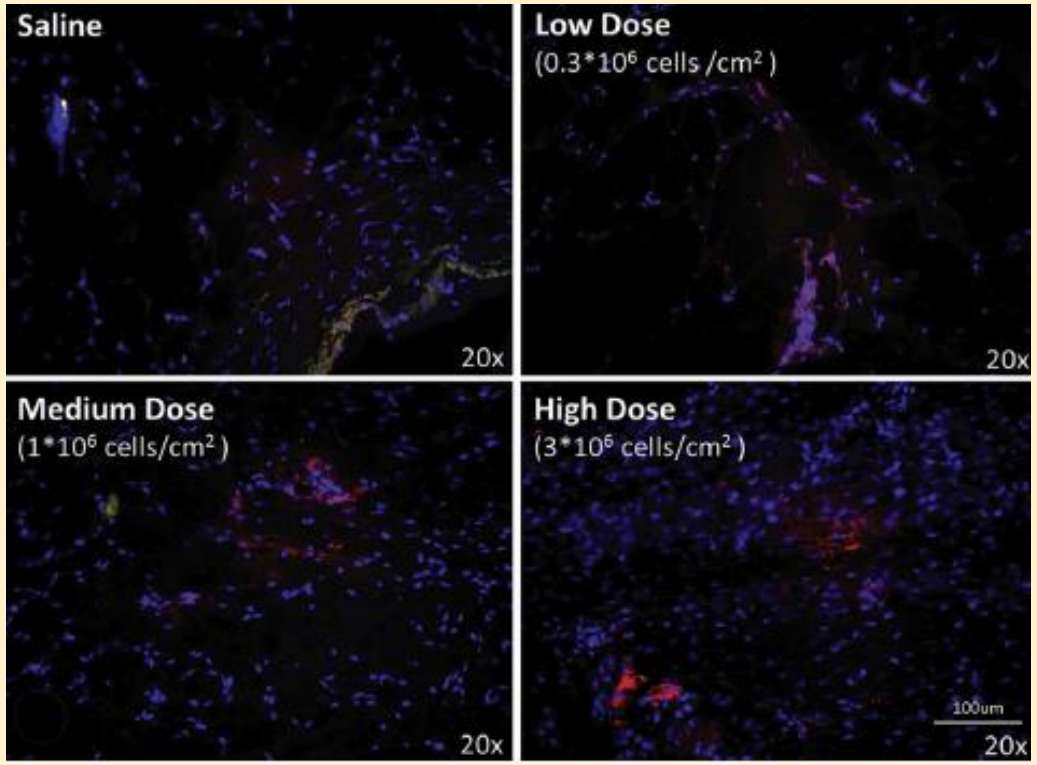
- PKH26-labeled ASC in deep neodermis
- 1 and 2 wk postoperative
- Magnification of 20×
- red (PKH 26)
- Blue (4’,6-diamidino-2-phenylindole)

Results

Cell tracking



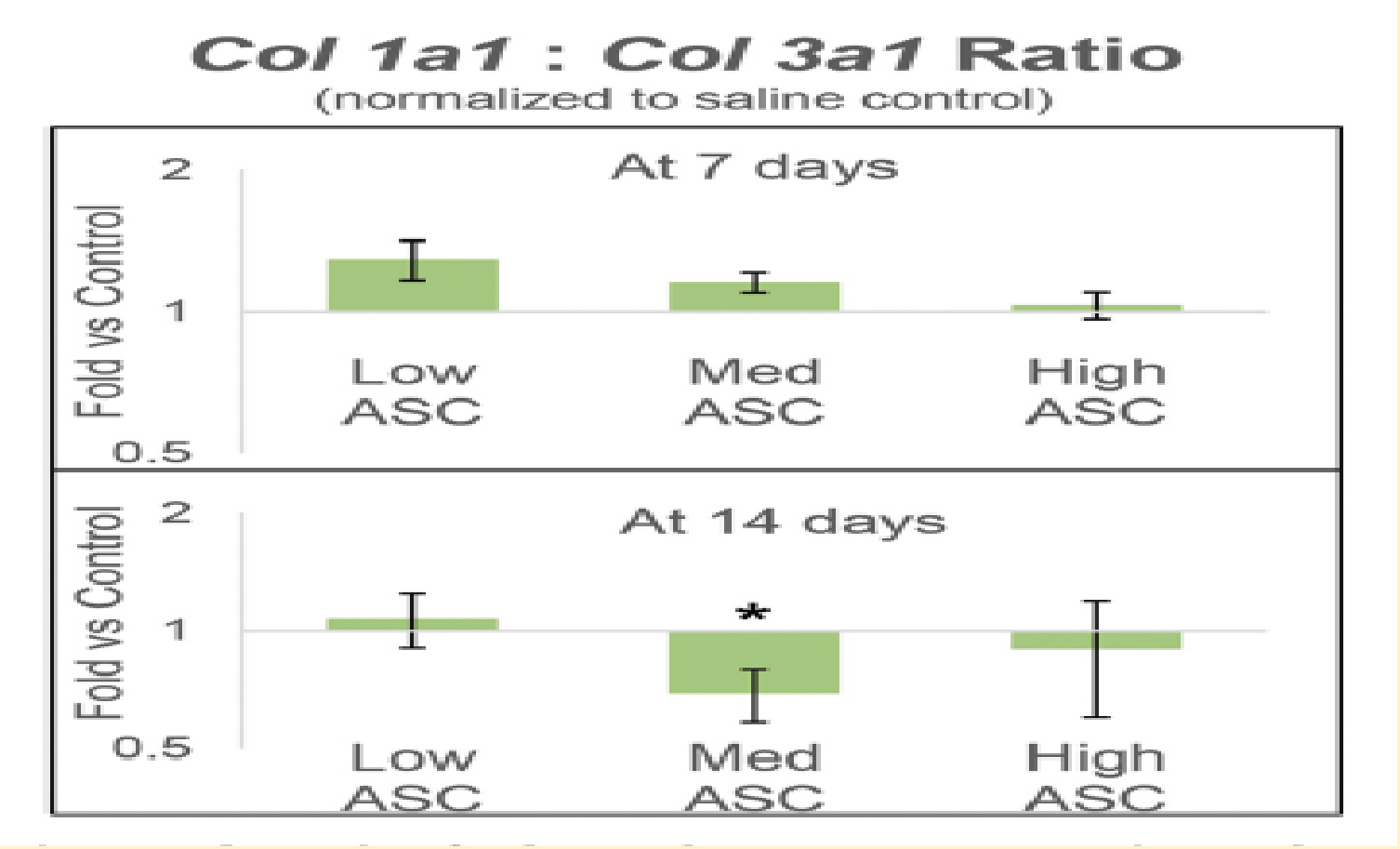
1 wk postoperative



at 2 wk

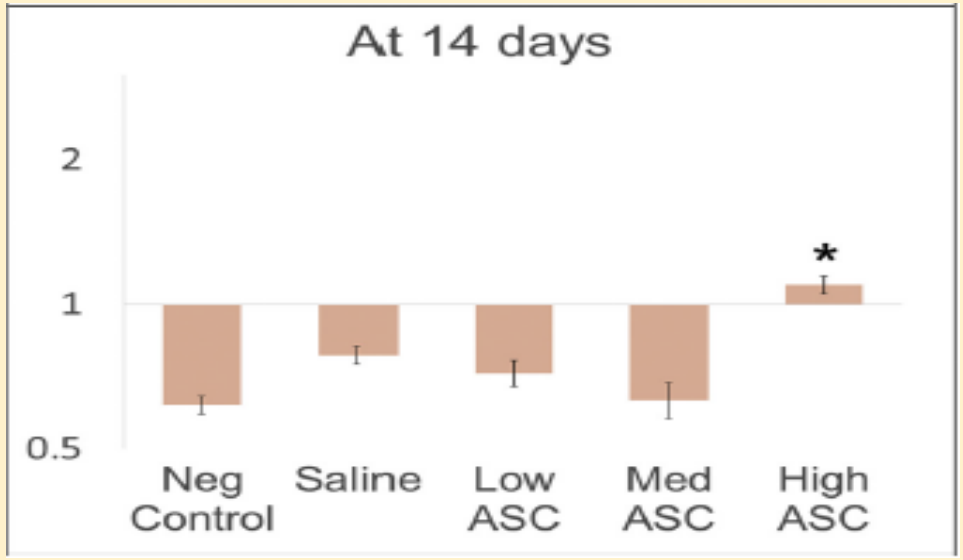
Results

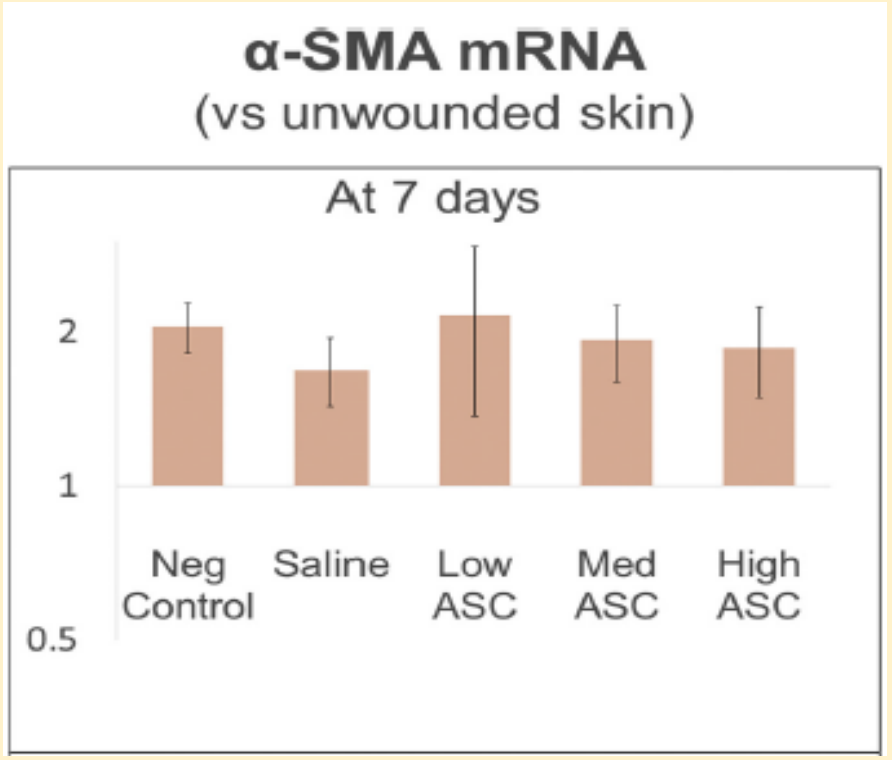
Quantitative RT-PCR



Results

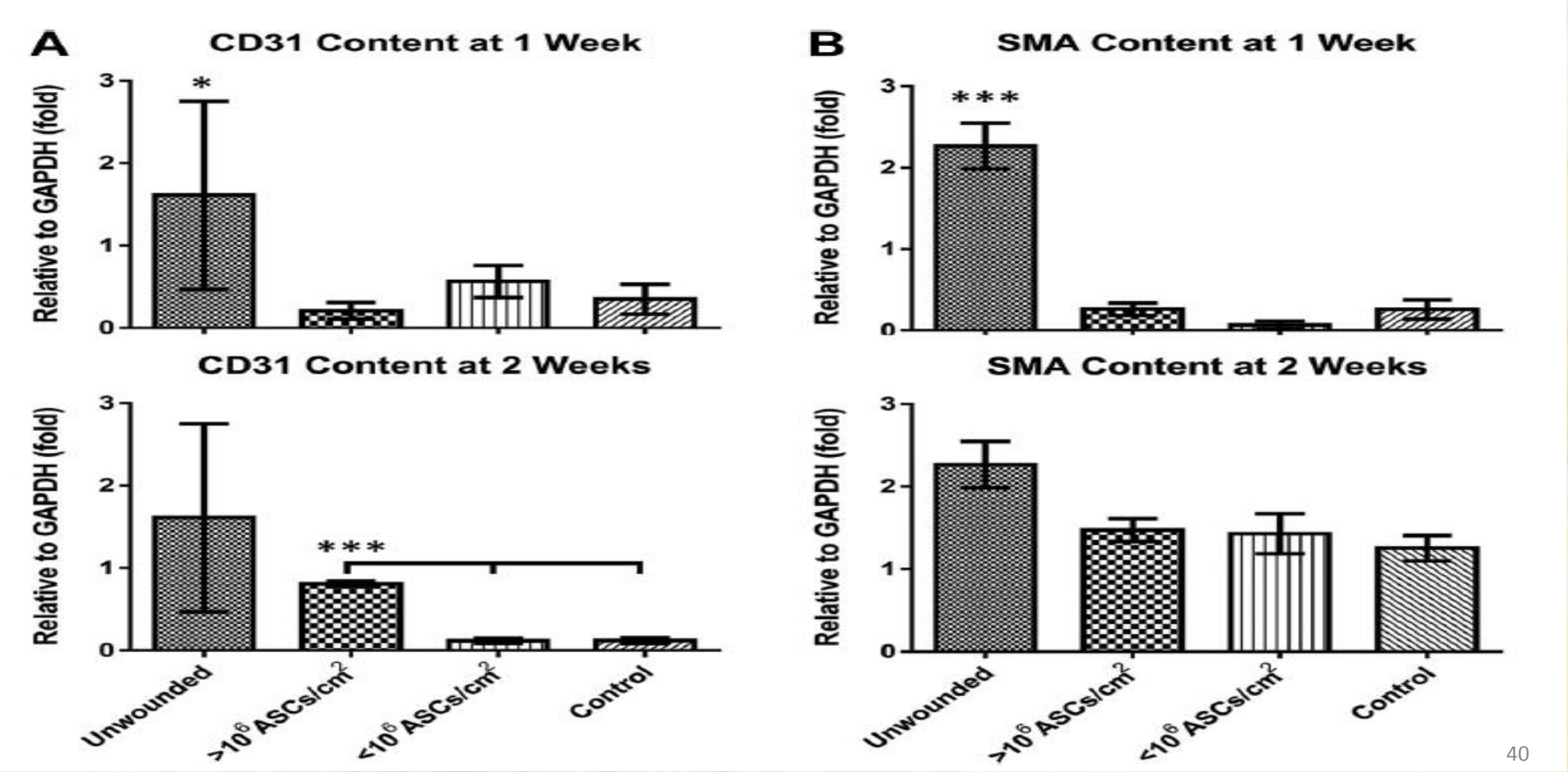
Quantitative RT-PCR and western blot





Results

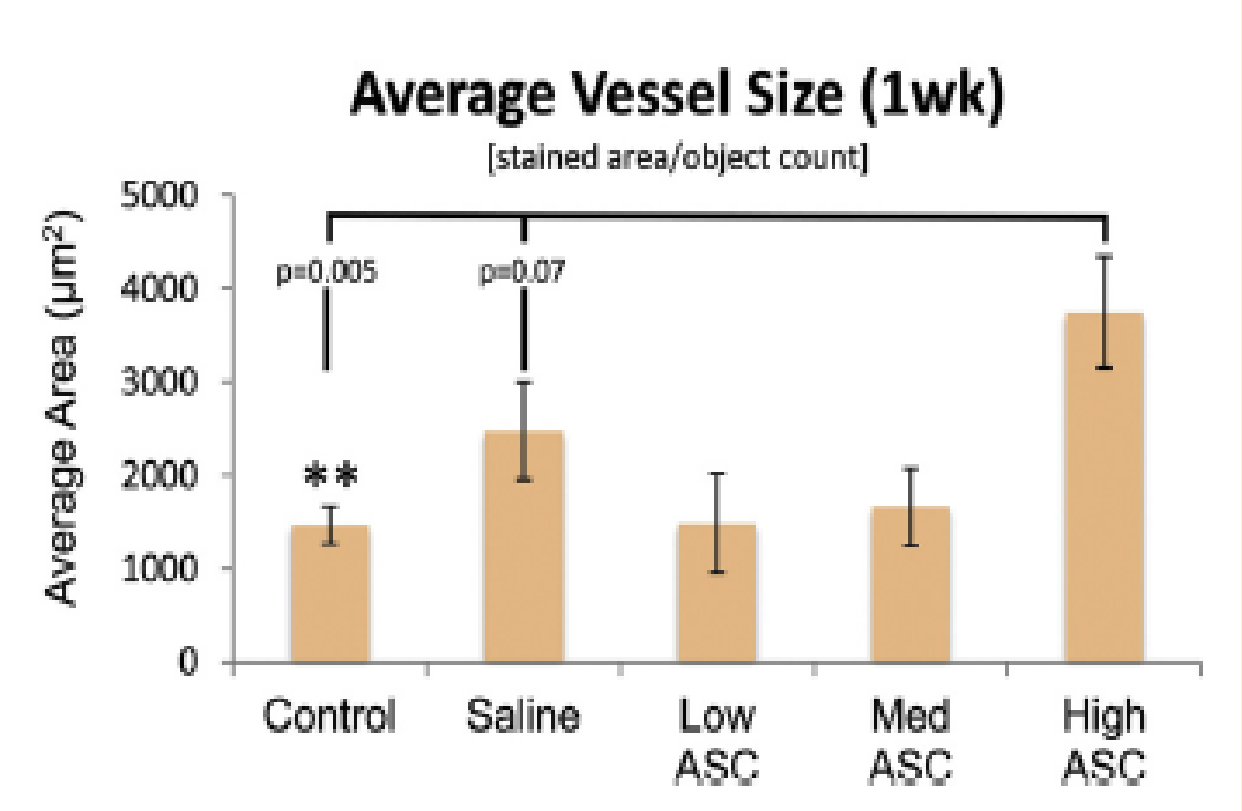
Western Blot



Results

Immunohistochemistry

- Meta morph software
- Per-pixel quantification



Discussion

ASC

- Well tolerated (if cultured)
- persist in the wound for at least 2 wk.
- the transient paracrine mediators do not survive long term
- reduce histocompatibility antigen presentation.
- ability to use in large animals:

➢the establishment of standardized cell banks

➢The flexibility of adipose stem cell-based treatments

Discussion

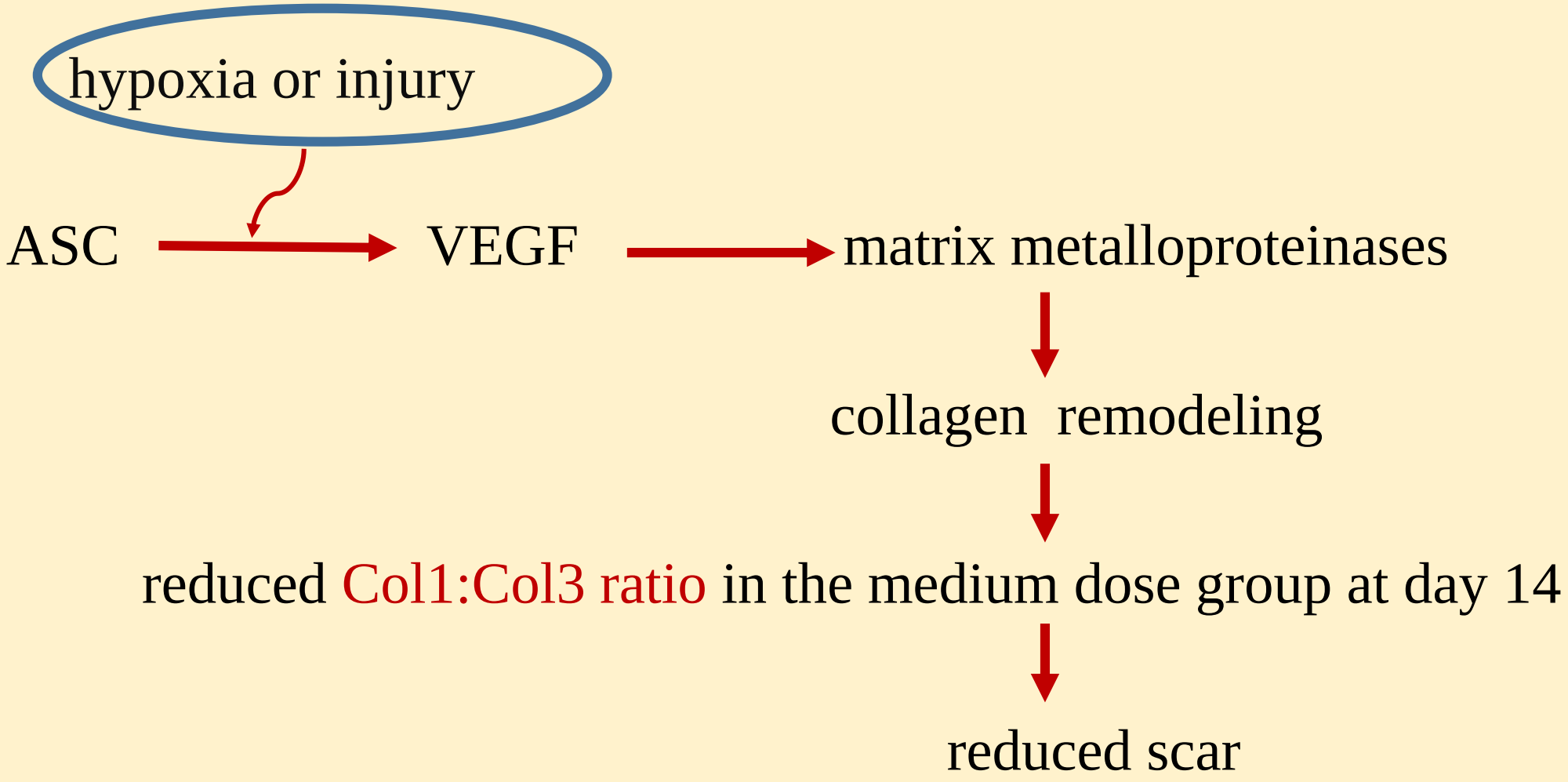
- did not significantly reduce time to epithelial coverage,
- high dose ASC enhance the rate of wound contraction
- enhanced neodermal thickness at 7 d.(reduce infection risk)

Discussion

ASC Dose dependent improvements

- CD31
- Col1:Col3 ratio
- α-SMA
- average vessel size

Discussion


hypoxia or injury
ASC
VEGF
matrix metalloproteinases
collagen remodeling
reduced Col1:Col3 ratio in the medium dose group at day 14
reduced scar

Discussion

α- SMA

- high dose group at 2 wk → ⇡ α- SMA

  consistent with:

  the enhanced wound contraction between day 10 and 14

- a reliable marker for myoepithelial cells' contractile activity.

- ASC → TGF-β → ⇡ α- SMA

Discussion

Limitations

- only acute phase of wound healing
- did not follow animals beyond 2 wk
- 4cm wounds are not a critical size defect
- Many of the effects only occurred at doses of 3 million cells/cm2